indicating a gradual build up during the period of their decline in the rice seedlings.

Baliothrips holorphnus (Karny) very closely allied to *Baliothrips biformis*, is another potential pest species, (sporadically noted in paddy, but more abundant in the growing seedlings of maize). Evidence of a distinct correlation between the degree of infestation of this species in young growing maize as well as in the abundant weeds, *Borreria hispida* and *Echinochloa colona*, is indicated in Fig. 2.

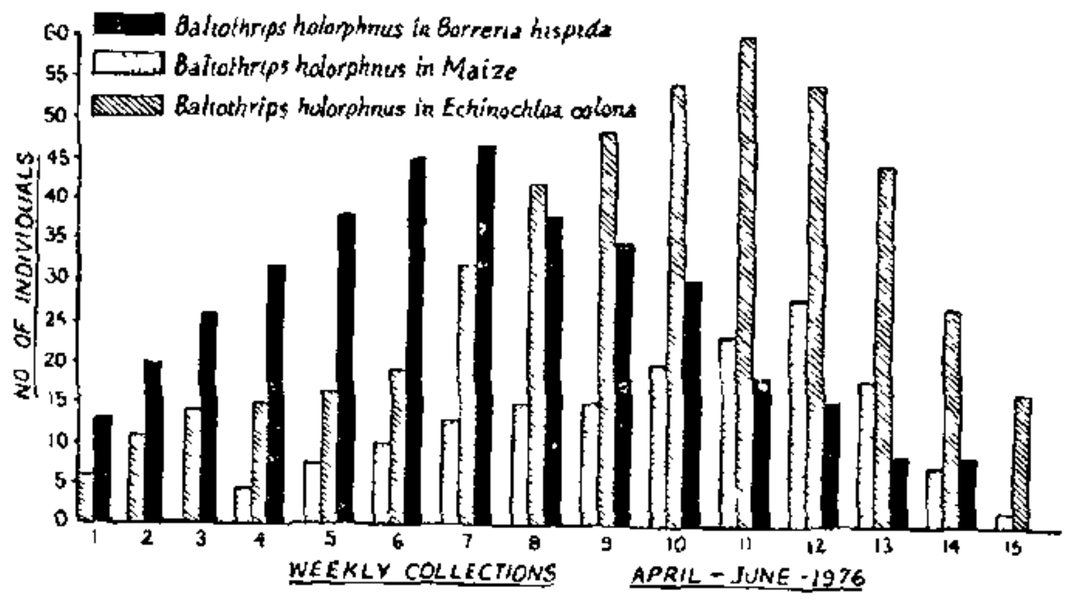

FIG. 2. Trends of infestation of *Baliothrips holorphnus* in maize, *Borreria hispida* and *Echinochloa colona*.

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* Zur Strassen describes this as a new species *Chloethrips blandus* retaining the generic name *Chloethrips* Priesner.

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EFFECT OF pH ON THE CELLULAR LOCALIZATION OF Δ^5 3β-HYDROXYSTEROID DEHYDROGENASE IN THE TESTIS OF THE SKINK, *MABUYA CARINATA* (SCHN.)

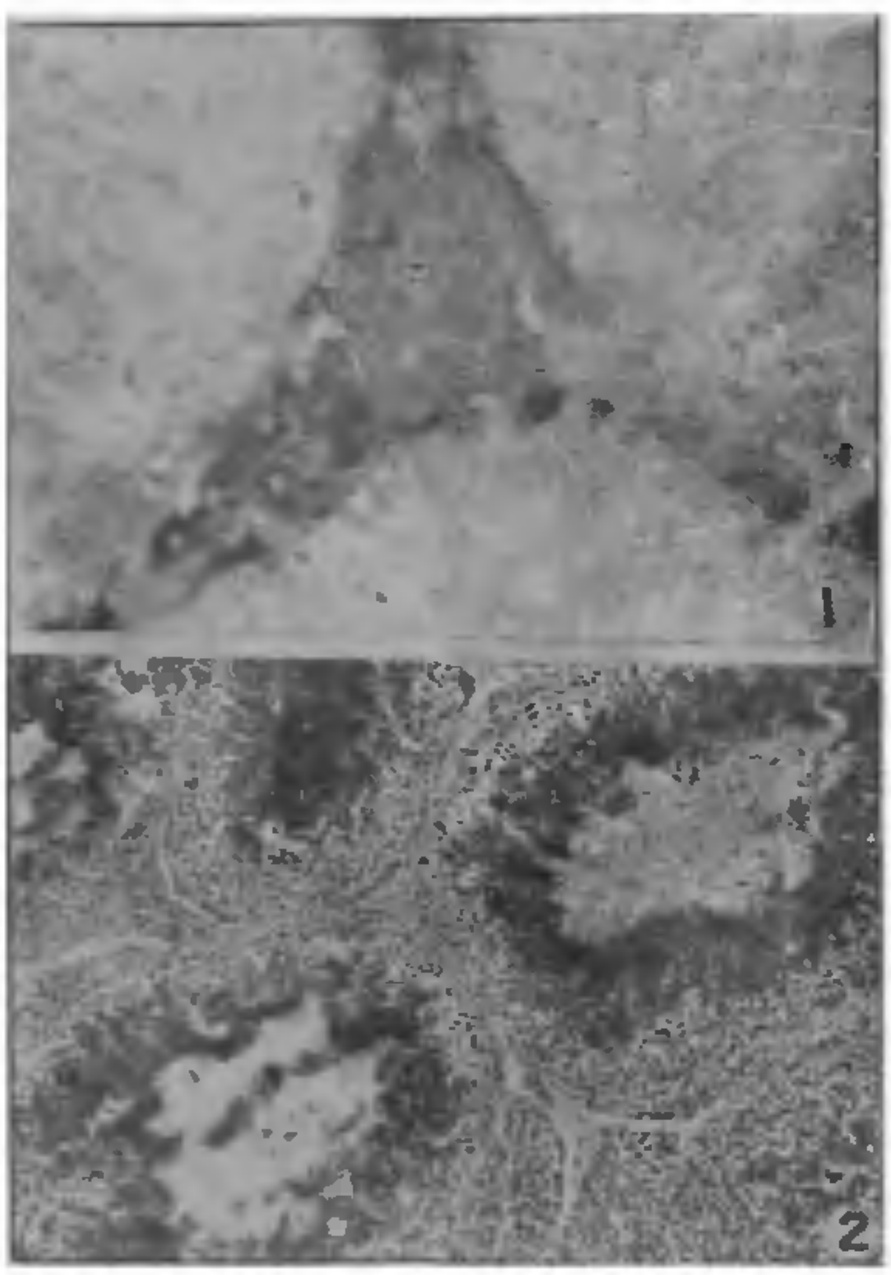
WATTENBERG (1958)[1] was the first to demonstrate the enzyme Δ^5 3β-Hydroxysteroid dehydrogenase (Δ^5 3β-HSDH) histochemically in steroidogenic tissues. Since then, this technique has been widely used as an important tool in the detection of cellular sites of steroidogenesis. The technique involves incubating frozen tissue sections in a buffered medium containing suitable hydroxysteroid substrate, appropriate co-factor and a tetrazolium salt as the final hydrogen acceptor. In the course of our investigations, on the testis of the skink, *Mabuya carinata* (Schn.), we found that the enzyme could be localized in the seminiferous tubules but not in the interstitial cells[2] at pH 7·5 according to the procedure of Baillie *et al.*[3]. But the Leydig cells in the testis of the garden lizard, *Calotes versicolor*[4] and the monitor, *Varanus monitor*[5] gave satisfactory results with the same procedure. This prompted us to undertake a detailed investigation into the kinetics of the localization of Δ^5 3β-HSDH selectively in the interstitial cells of Leydig taking ino account (i) the pH of the buffer, (ii) the steroid substrate concentration, (iii) the co-factor concentration, and (iv) the concentration of the tetrazolium salt.

Testes from the sexually mature skinks during the breeding season were frozen and sections were cut at 16 μ in a cryostat maintained at – 20° C. The sections were thawed for a moment and incubated (with or without prior acetone treatment to fix the enzyme and to remove free lipids) for 1 hr at 37° C. The incubating media consisted of the steroid substrate, Dehydroepiandrosterone or Pregnenolone (0·25 mg to 2·5 mg/ml), β-NAD (0·5 mg to 3 mg/ml) and nitroblue tetrazolium (0·25 mg to 1·5 mg/ml) in 0·1 M Tris buffer at various pHs from 6·5 to 8·5. Controls were incubated omitting the substrate or treating the section in boiling water. After incubation, the sections were fixed in 10% neutral formalin and mounted in glycerine jelly or PVP medium. The enzyme activity was visually quantitated based on the amount of formazan deposition.

The concentration of the co-factor and of the tratrazolium salt were not so critical within the ranges we have used so far as the site of enzyme localisation is concerned. But pH had a profound influence on the activity of the enzyme and on the cellular site of its localization. At precisely pH 8·0, the enzyme showed highest activity and could be selectively localized in the Leydig cells (Fig. 1), whereas at pH 7·5 formazan deposition could be clearly seen mainly in the seminiferous tubules (Fig. 2), and no formazan deposition could be observed at other pH values used. The following alteration of Baillie *et al.*'s incubating medium gave best results for this tissue; the steroid substrate (0·4 mg/ml), β-NAD (1·5 mg/ml), NBT (0·5 mg/ml) in 0·1 M Tris HCl buffer (pH 8·0).

Biosynthetic pathway of almost all the hormonally active steroids involves the conversion of Δ^5 3β-hydroxysteroids into their ketoforms and the enzyme system carrying out this reaction has been called Δ^5 3β-hydroxysteroid dehydrogenase which preferentially utilises NAD as the coenzyme[6,7]. In the histochemical reaction, if this enzyme is present in the tissue, it oxidises the steroid substrate and the hydrogen is finally accepted by tetrazolium salt which gets reduced to the coloured, insoluble formazan formed at the site of reaction in the tissue section. $NADH_2$-tetrazolium reductase or

$NADH_2$-diaphorase catalyses the latter part of the reaction. Hence the presence and distribution of $NADH_2$-diaphorase in the tissue is one of the limitations in the histochemical localization of Δ^5 3β *HSDH*, and it should either be ubiquitous or at least be present at the site of Δ^5 3β-HSDH[3]. The possible explanations for the formazan observed in the seminiferous tubules in *M. carinata* are the following: (i) a false positive reaction or "nothing dehydrogenase" effect which may be pH dependent, (ii) non-specific reduction of the tetrazolium, probably non-enzymatic, (iii) a broad-spectrum alcohol dehydrogenase in the seminiferous tubules capable of oxidising the steroid substrate[8], (iv) differential histochemical threshold of the localisation of Δ^5 3β-HSDH in the seminiferous tubules and the interstitial cells of the testis, selectively demonstrable at different values of pH[2].



FIGS. 1-2. Fig. 1. Cross-section of the testis of *M. carinata* showing Δ^5 3β-HSDH activity in the interstitial cells at pH 8·0 (8 × 40). Fig. 2. Cross-section of the testis showing the formazan mainly in the seminiferous tubules at pH 7·5 (8 × 10).

Δ^5 3β-HSDH which has been demonstrated in the interstitial cells of many reptiles[9-12], and *in vitro* biochemical conversions[13,14] strongly point out, the involvement of Leydig cells in the interstitium of the reptilian testis in the biosynthesis of androgens. The demonstration of this enzyme, in interstitial cells of the testis in *M. carinata*, hardly leaves any doubt as to their potentiality in steroidogenesis, though involvement of seminiferous tubules at least partly cannot be ruled out

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INDUCED PARASITISM OF A FREE-LIVING NEMATODE, *ACROBELOIDES APICULATUS* (THORNE, 1925) IN DIPTEROUS LARVAE

STEINER AND BUHRER[2] and recently Wasilewska and Webster[4] have reviewed the importance of free-living nematodes as disease factors of man and his crops. *Acrobeloides apiculatus* (Thorne, 1925) Thorne, 1937, a free-living nematode is of frequent occurrence in Aligarh soils. The latter also usually contains dipterous larvae of an unidentified species. The nematodes were raised *in vitro* on Nigon's agar medium (bacteriological). Although in nature the nematodes were never found to parasitise the dipterous larvae, but when introduced artificially by making a small incision at the anterior end of larvae they started to live in the coelom as parasites without themselves showing any obvious signs of discomfort. When gravid females of *A. apiculatus* from the culture were introduced within the body cavity of dipterous larvae (Fig. 1) they failed to lay eggs which then began to develop inside the uterus (intra-uterine egg development). The hatching also took place within the uterus and the juveniles of *A. apiculatus* began to feed upon the body tissues of the mother which ultimately died and the juveniles were liberated in the coelom of the dipterous larvae.